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# LASER-BASED INHIBITION OF SMOOTH MUSCLE CELL HYPERPROLIFERATION

## Background of the Invention

The field of the invention is inhibition of smooth muscle cell proliferation.

Smooth muscle cell hyperproliferation is one of the primary underlying caused of restenosis (re-obstruction of a vascular passage following vascular surgery). Restenosis occurs within 6 months of nonsurgical revascularization in 15% to 43% of the cases (Block et al., in *Coronary Heart Diseases: Prevention, Complications, and Treatment*, Connor et al., (eds) Lippincott, Philadelphia, pp. 405-418, 1985; Holmes et al., *Am. J. Cardiol.* 53:77c, 1984; Mabin et al., *Circulation* 71:754, 1985). Recurrent myocardial ischemia is observed in many patients suffering restenosis. Asymptomatic restenosis, which may result in sudden death is observed in some cases (Levine et al., *Am. J. Cardiol.* 55:673, 1985). Restenosis is observed whether angioplasty is performed by balloon catheter, laser ablation, or thermal ablation.

The degree of restenosis may depend on the location and morphologic features of the original lesion, the degree of dilation during angioplasty, and the degree of damage to the arterial wall during angioplasty. While smooth muscle cell hyperproliferation is thought to be the primary cause of restenosis, unsuitable dilation during angioplasty, platelet activation, and coagulation activation may also be involved (Cos et al., *Can. Med. Assoc. J.* 134:1129, 1986).

Endothelial desquamation caused by injury to the arterial wall may be an important factor in inducing restenosis. The loss of endothelial cells exposes the underlying connective tissue to factors present in the

blood, including platelet derived growth factor and other mitogens, which may be the proximal cause of smooth muscle cell hyperproliferation and concomitant extracellular matrix deposition. Disruption of endothelium can also lead to platelet deposition and activation of the coagulation system, steps which can result in formation of an occlusive thrombus.

There have been attempts to treat restenosis by interfering with platelet deposition or thrombus formation. Acetylsalicylic acid pre-treatment has been shown to reduce platelet accumulation in patients who have undergone coronary angioplasty (Cunningham et al., *Radiology* 151:487, 1984). A placebo controlled study in 376 patients demonstrated that while an aspirin-dipyridamide "antiplatelet regimen before and after PTCA (percutaneous transluminal coronary angioplasty) did not reduce the six-month rate of restenosis after successful coronary angioplasty, it markedly reduced the incidence of transmural myocardial infarction during or soon after PTCA" (Schwartz et al., *N. Engl. J. Med.* 318:1714, 1988). Heparin has been proposed as a treatment for restenosis. Heparin has been shown to reduce platelet accumulation on the denuded neointima (Mustard, *Ann. R. Coll. Physicians Surg. Can.* 14:22, 1981). A study found that intravenous heparin in doses large enough to cause continuous anticoagulation reduced myointimal thickening in rats whose carotid arteries had been injured (Clowes et al., *Nature* 265:625, 1977). An *in vivo* study found that heparin inhibits smooth muscle cell proliferation which occurs after denudation of endothelium by air-drying the rat carotid artery; this effect does not depend on anticoagulant activity (Guyton et al., *Circ. Res.* 46:625, 1980). *In vitro* studies of cultured rat smooth muscle cells demonstrated that heparin, in either its high anticoagulant form or its non-anticoagulant form,

significantly inhibits cell proliferation (Hoover et al., *Circ. Res.* 47:578, 1980).

Smooth muscle cell proliferation following damage to the arterial wall may involve platelet-derived growth factor. Triazolopyrimidine (trapidil), an inhibitor of platelet-derived growth factor-induced cellular proliferation, has been shown to prevent restenosis following balloon angioplasty in rabbits having experimentally induced atherosclerosis. However, "[t]he variability of the response of atherosclerotic rabbit iliac arteries to balloon injury makes this model less than ideal" (Lin et al., *Circulation* 81:1089, 1990).

It has been proposed that a local angiotensin system may participate in regulation of vascular response to injury (Powell et al., *Science* 245:186, 1989). Cilazapril, an inhibitor of angiotensin-converting enzyme, has been shown to reduce restenosis in rats which had been subjected to endothelial denudation by balloon catheterization (Powell et al., *supra*).

Photoreactive psoralens, which are used in the present invention described below, are known to intercalate in double-stranded DNA and, upon absorbing ultraviolet light, form covalent linkages to pyrimidine bases (monoadducts) and, less often, cross-linkages between pyrimidines on opposite strands (cross-links). This modification of DNA is thought to be the basis for the inhibition of DNA synthesis observed in several cell types upon irradiation in the presence of a photoreactive psoralen derivative. Psoriasis is caused by hyperplasia of the epidermis, and the ability of light-activated psoralens to block DNA synthesis provides a rationale for the effectiveness of psoralens in treatment of this condition.

## Summary of the Invention

In general, the invention features a method of inhibiting smooth muscle cell proliferation in a patient. The method includes the steps of: (a) administering a photoreactive psoralen derivative to the patient so that the psoralen derivative is taken up by smooth muscle cells of the patient, and (b) irradiating the smooth muscle cells whose proliferation is to be inhibited with light, of an approprite wavelength for a period of time and at an intensity sufficient to inhibit proliferation.

In preferred embodiments, the proliferation is caused by vascular injury; the proliferation is caused by vascular surgery; the proliferation is caused by angioplasty. In even more preferred embodiments, the angioplasty is balloon angioplasty; is laser angioplasty; and is mechanical angioplasty.

In another preferred embodiment, the psoralen derivative is monofunctional. In even more preferred embodiments, the monofunctional derivative is kallein, is 3-carbethoxypsoralen; is angelicin; and is an angular furocoumarin.

In another preferred embodiment, the psoralen derivative is bifunctional. In more preferred embodiments, the bifunctional psoralen derivative is 8-methoxypsoralen; is 5-methoxypsoralen; and is trimethyl psoralen.

"Psoralen derivatives", as used herein, and substitutions which allow covalent binding to DNA. By "photoreactive psoralen derivative" is meant a compound having the basic furan ring structure of psoralen (7H-Furo[3,2-g][1]benzopyran-7-one) and groups on this ring structure (e.g., at the 4, 5 or 8 positions) such that the molecule, upon exposure to light of an appropriate wavelength, can form a covalent bond with at least one pyrimidine.

By "monofunctional psoralen derivative" is meant a psoralen derivative which can only form a DNA monoadduct, i.e., it can only be covalently bound to a single pyrimidine. By "bifunctional psoralen derivative" is meant a psoralen derivative having functional groups which form a monoadduct or a DNA cross-link, i.e., it can be covalently bound to two pyrimidine molecules simultaneously.

The method of the invention provides a treatment for inhibition of unwanted smooth muscle cell proliferation, e.g., inhibition of restenosis. The method employs compounds that have been well studied and are know to be safe for treatment of other conditions. The method also provides a means by which treatment can be specifically targeted to the regions requiring treatment.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

## Detailed Description

The drawings will first briefly be described.

### Drawings

Fig. 1 is a graph depicting the effect of ultraviolet light and 8-methoxypsoralen on thymidine uptake by hyperproliferative smooth muscle cells;

Fig. 2 is a graph depicting the effect of ultraviolet light and 8-methoxypsoralen on succinate dehydrogenase activity of hyperproliferative smooth muscle cells.

### Psoralens

The invention provides a method for reducing unwanted smooth muscle cell proliferation by means of light-activated drugs, psoralens. As illustrated below, 8-methoxypsoralen, upon exposure to ultraviolet light,

inhibits proliferation of hyperproliferative smooth muscle cells without decreasing cell viability. These results demonstrate that psoralen derivatives and ultraviolet light can be used to suppress smooth muscle cell proliferation despite the presence of high concentrations of growth factors such as platelet-derived growth factor. Because inhibition of cell proliferation by psoralen persists for only several weeks, there should be few negative long term consequences of treatment according to the invention. Smooth muscle proliferation is normally regulated by the endothelial layers covering the smooth muscle cells. Since this layer regenerates within one or two weeks, the relatively short duration of psoralen-induced proliferation inhibition can inhibit restenosis while permitting the normal vascular milieu to be re-established. Since endothelial regeneration normally occurs from the areas adjacent to the site of injury, irradiation of only the site of injury with the appropriate illumination device will inhibit smooth muscle cell proliferation but not inhibit re-establishment of endothelial cells by migration and proliferation of endothelial cells from adjacent regions. Psoralen is a particular isomer of furocoumarin (molecules in which a furan ring is fused to a benzo-α-pyrone). A number of psoralen derivatives are derived from plants and have long been used in combination with irradiation for the treatment of vitiligo and psoriasis in a technique known as photochemotherapy. In the absence of irradiation many psoralens are chemically and biologically inert.

Psoralen derivatives useful in the method of the invention are the photoreactive derivatives which, when irradiated with light in the violet to ultraviolet range (e.g. 210-410 nm), will inhibit smooth muscle cell proliferation, and which are substantially inert

physiologically in the absence of ultraviolet light. The psoralen derivatives used in the method of the invention preferably have a therapeutic index for killing (toxicity of the psoralen derivative when irradiated at the therapeutic wavelength: toxicity of the psoralen derivative when not irradiated) of at least 500. Presented below is a simple *in vitro* assay for inhibition of smooth muscle cell proliferation. Psoralen derivatives which are known to photoreact with DNA and those derivatives known to be useful for treatment of psoriasis are potentially useful in the method of the invention. Psoralen derivatives commonly used for photochemotherapy of psoriasis and vitiligo include methoxsalen (8-methoxypsoralen), bergapten (5-methoxypsoralen), and trioxsalen (4,5′,8-trimethylpsoralen) (de Wolff et al., *Clin. Pharmacokinetics*, 11:62, 1986; Gupta et al., *J. Amer. Acad. Derm.* 17:703, 1987). A number of other psoralen derivatives (4′-hydroxymethyl-4,5′,8-trimethylpsoralen, 4′-methoxymethyl-4,5′,8-trimethylpsoralen, and 4′-aminomethyl 4,5′,8-trimethylpsoralen hydrochloride) are known to photoreact with nucleic acids (Issacs et al., *supra*). A convenient *in vitro* assay for DNA binding is described by Issacs et al. (*Biochemistry* 16:1058, 1977). In addition, rules have been developed for identifying those psoralen derivatives likely to be photoreactive. For example, electron donating moieties such as methyl groups at the 5′ or 8 position increases activity, while electron withdrawing groups such as hydroxyl, cyanol, nitro or acetyl amino groups at these positions decrease activity (Scott et al., *supra*). Addition of electron donating groups at the 3,4, or 4′ positions decrease activity, while electron donating groups at these positions have the opposite effect. Rules have also been developed for identifying derivatives that are likely to

react with DNA (Issacs et al., *supra*). Such rules can be used to design and select psoralen derivatives which can then be screened using the smooth muscle cell proliferation assay described below.

Example

Smooth muscle cells obtained from New Zealand White Rabbits were plated at $10^4$ cells/ml in Dulbecco's Modified Eagle's Medium (DMEM) including 2% fetal calf serum. After 24 hours the medium was removed and replaced with 8-methoxypsoralen at 8 $\mu$g/ml (dissolved in ethanol and then diluted with DMEM); control cells received no treatment. After 5 min the cells were treated with 365 nm ultraviolet light at various intensities (1 kW solar simulator with copper and cobalt sulfate filters) for a period sufficient to deliver the appropriate dose of radiation (usually 1 sec-4 min). The 8-methoxypsoralen was removed after irradiation and replaced with DMEM including 20% fetal calf serum (to stimulate hyperproliferation). Cell proliferation was assayed 48 hours later by a thymidine uptake assay and by an MTT assay. As shown in Fig. 1, thymidine incorporation decreased with increasing irradiation energy. Control cells irradiated with the highest energy light 200 mJ were unaffected.

Cell viability was determined 48 hours after irradiation using previously described assay for succinate dehydrogenase activity (*J. Immunol. Methods* 89:271, 1986). As shown in Fig. 2, succinate dehydrogenase activity of 8-methoxypsoralen treated cells was unaffected even at the highest irradiation energies.

Therapy

To reduce restenosis following angioplasty, a photoreactive psoralen derivative is administered prior to angioplasty and the area subjected to angioplasty or vascular surgery is then irradiated with light of a wavelength and intensity that will activate the particular psoralen derivative being used. The administration of psoralen may be oral, intravenous or intracoronal. Local administration can be achieved using a fluid filled catheter for both irradiation (as described in Gregory et al., PCT Application PCT/US88/04740) and delivery of the psoralen derivative to the arterial region to be treated. The dosage required depends on a number of factors, including the mode of administration, the concentration required for inhibition of smooth muscle cell proliferation, and the pharmacokinetic behavior of the particular psoralen derivative used. The clinical pharmacokinetics of psoralen derivatives has been extensively studied (de Wolff et al., *Clin. Pharmacokinetics* 11:62, 1986). In treatment of psoriasis, the standard oral dosage of 8-methoxypsoralen is 0.5 to 0.7 mg/kg.

Irradiation of the area to be treated can be carried out in conjunction with angioplasty. This requires the use of a catheter which can deliver ultraviolet light of the appropriate wavelength by means of an optical fiber or some other suitable method known to those of ordinary skill in the art, e.g., a fluid core catheter (Gregory et al., *supra*), a bare optical fiber or a laser balloon.

Wavelengths of 300-410 nm and fluences of up to 100 J/cm$^2$ may be used to irradiate psoralen treated cells for the purpose of inhibiting cell proliferation. The light fluence used is an important consideration, and selection of an appropriate fluence depends on such

factors as drug concentration, accessibility of the cells being irradiated, and whether the formation of primarily monoadducts or cross-links is desired. At psoralen concentrations in the range of 1 $\mu g/ml$, it is possible to effectively inhibit cell proliferation using 330 nm light at fluences as low as 0.1 $J/cm^2$. Of course, the actual fluence required may be substantially higher since cells and other material interposed between the light source and the cells being targeted will absorb some of the energy. The action spectra of many psoralens indicate that higher fluences are required when longer wavelength light is being used. Thus, the <u>effective</u> dose for 380 nm light might be 1 to 10 $J/cm^2$ (or less) while higher fluences might be required at longer wavelengths.

Both monofunctional and bifunctional psoralen derivatives are useful in the method of the invention. The bifunctional derivatives more effectively inhibit cell proliferation than the monofunctional derivatives since they can form DNA cross-links, as well as the less toxic DNA monoadducts. Cross-link formation requires the absorption of two photons; absorption of the first photon leads to monoadduct formation while absorption of the second photon converts the monoadduct to a cross-link. In many cases the wavelength required for conversion of the monoadduct to a cross-link (320-380 nm) is shorter than that required for formation of the monoadduct (380-410 nm). Irradiation can be performed twice, once at a relatively long wavelength to form monoadducts and a second time at a shorter wavelength to form cross-links. Drug concentration and fluence are important variables for determining the ratio of monoadduct to cross-link formation. When the drug concentration is relatively low, cross-link formation requires higher fluences since the probability of any one drug molecule absorbing two photons is lower than at higher drug concentrations.

In selecting an appropriate wavelength for treatment, it should be recognized longer wavelengths may cause fewer side effects and will generally penetrate deeper than shorter wavelengths. Thus, selection of an appropriate wavelength for irradiation must balance the desire to form more potent cross-links with the desire for effective penetration and safety.

Claims

1. Use of a photoreactive psoralen derivative in the preparation of a medicament for inhibiting smooth muscle cell proliferation in a patient by administering said photoreactive psoralen derivative to said patient so that said psoralen derivative is taken up by smooth muscle cells of said patient and then irradiating the smooth muscle cells whose proliferation is to be inhibited with light of an appropriate wavelength, for a period of time and at an intensity sufficient to inhibit proliferation.

2. The use of claim 1 wherein said proliferation is caused by vascular injury.

3. The use of claim 2 wherein said vascular injury is caused by vascular surgery.

4. The use of claim 3 wherein said vascular injury is caused by angioplasty.

5. The use of claim 4 wherein said angioplasty is balloon angioplasty.

6. The use of claim 4 wherein said angioplasty is laser angioplasty.

7. The use of claim 4 wherein said angioplasty is mechanical angioplasty.

8. The use of claim 1 wherein said psoralen derivative is monofunctional.

9. The use of claim 1 wherein said psoralen derivative is bifunctional.

10. The use of claim 9 wherein said psoralen derivative is 8-methoxypsoralen.

11. The use of claim 9 wherein said psoralen derivative is 5-methoxypsoralen.

12. The use of claim 9 wherein said psoralen derivative is trimethylpsoralen.

13. The use of claim 8 wherein said psoralen derivative is kallein.

14. The use of claim 8 wherein said psoralen derivative is 3-carbethoxypsoralen.

15. The use of claim 8 wherein said psoralen derivative is an angular furocoumarin.

16. The use of claim 8 wherein said psoralen derivative is angelicin.

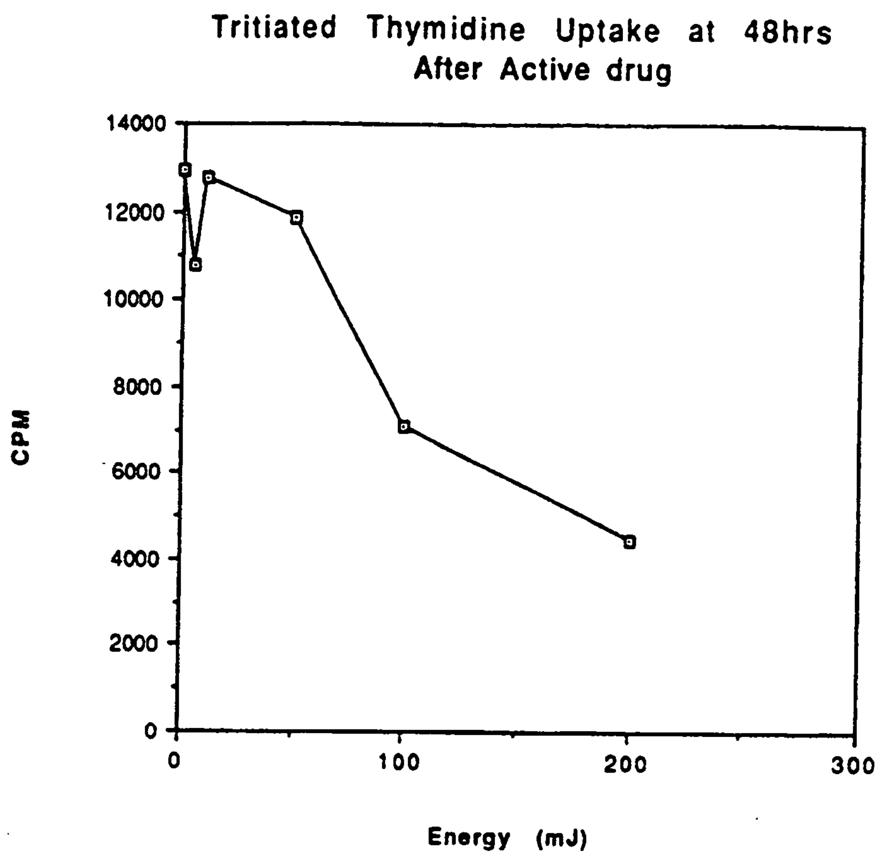
Tritiated Thymidine Uptake at 48hrs
After Active drug
14000
12000
10000
8000
6000
4000
2000
0
CPM
0
100
200
300
Energy (mJ)

Fig. 1

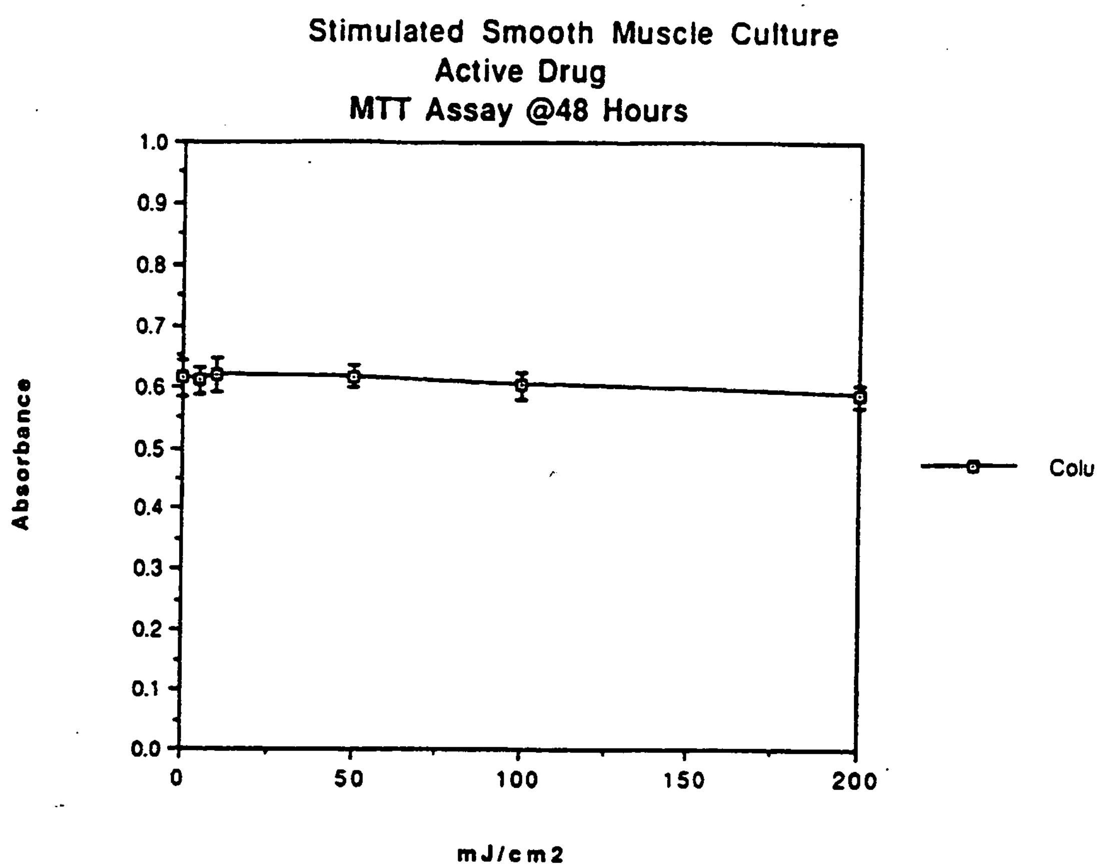
Stimulated Smooth Muscle Culture
Active Drug
MTT Assay @48 Hours
Absorbance
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
50
100
150
200
mJ/cm2
Colu

Fig. 2

# PATENT COOPERATION TREATY

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## DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT

(PCT Article 17(2)(a) and Rule 39)

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|---|---|---|
| Applicant's or agent's file reference 00786/063WO1 | IMPORTANT DECLARATION | Date of mailing *(day/month/year)* 26 JAN 1993 |
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| International Patent Classification (IPC) or both national classification and IPC<br>IPC (5): A01N 43/16, 47/10; A61K 31/27, 31/35<br>US CL: 514/455, 480 | | |
| Applicant<br>THE GENERAL HOSPITAL CORPORATION | | |

This International Searching Authority hereby declares, according to Article 17(2)(a), that **no international search report will be established** on the international application for the reasons indicated below.

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   - b. [ ] mathematical theories.
   - c. [ ] plant varieties.
   - d. [ ] animal varieties.
   - e. [ ] essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes.
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   - m. [ ] computer programs for which this International Searching Authority is not equipped to search prior art.

2. [X] The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:

   [ ] the description  [X] the claims  [ ] the drawings

3. [ ] The failure of the nucleotide and/or amino acid sequence listing to comply with the prescribed requirements prevents a meaningful search from being carried out:

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4. Further comments:
(See Attached)

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International application No.
PCT/US92/09149

4. Further Comments (Continued):

The claims are written as "use" claims, which do not comply with PCT Rule 6.3(a). Further, the claims do not comply with PCT Article 17(2)(a).